REVIEW ARTICLE

Kisspeptins: a multifunctional peptide system with a role in reproduction, cancer and the cardiovascular system

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Abstract

The pairing of the kisspeptins (KP) with the KISS1 (GPR54) receptor has received growing attention since the description of the receptor as a molecular switch for puberty. The role of KP and its receptor, GPR54, in puberty is the most exciting finding made in the field of reproductive biology since the discovery of Gonadotropin Releasing Hormone (GnRH) in 1970s. A significant body of evidence across several species now suggests that KISS1 (GPR54) activation is a critical point in the commencement of puberty, although further investigation is required to characterize the interaction between KP and GnRH cascade. Given such pivotal roles of kisspeptins and GPR54 as gatekeepers of reproductive function, and the proven ability of sex steroids to physiologically regulate this system, it is plausible that environmental compounds with ability to interfere oestrogen and/or androgen signaling (agonists or antagonists) may target the hypothalamic kiss-1/GPR54 system, thereby inducing functional alterations of the hypothalamic-pituitary-gonadal axis. Synthetic agonists targeting KISS1 (GPR54) may represent novel therapeutic agents for the treatment of hypogonadotrophic hypogonadism in some affected individuals. The diverse multifunctional nature of the KP is beginning to unravel. The unexpected role of these peptides in puberty has raised a number of important questions that remain to be answered. Hippokratia 2008; 12 (4): 205-210

Key words: kisspeptins, KISS1 receptor, puberty, polycystic ovary syndrome, cancer, cardiovascular system

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The kisspeptins (KP) were originally identified in 1996 from a metastasis suppressor gene, kiss-1, in malignant melanomas[1]. Predicted KISS1 protein (the receptor of KP) consists of 145 amino acids and shares no similarity with other known proteins. Examination of the peptide sequence reveals a number of potentially important motifs for several post- translational modifications.

Initially, the largest cleavage product, KP-54, was identified for its ability to suppress metastatic potential in human melanoma cells. Its expression also resulted in suppression of melanoma metastasis in athymic nude mice and it was therefore termed metastin.

Three biologically active cleavage peptides of the kiss-1 gene product have been isolated from human placenta: KP-54, KP-13 and KP-10. These peptides are called kisspeptins[2,3].

Initial molecular localization has revealed limited expression in both the periphery and the brain, with particularly high expression in the placenta, although variation in reported expression exists[1,3,4].

The present review summarizes published data on the physiology and potential interactions of KP and the KISS1 receptor and their possible role as an unexpected molecular switch for puberty.

KP-KISS1 (GPR 54) system

The novel receptor KISS1 (previously designated GPR54, AXOR12 or Hot7T7T175) was isolated in 1999 by a degenerate PCR search of rat brain[5]. It shares significant homology with galanin receptors (44-45%). The GPR54 gene maps to chromosome 19p13.3, contains 5 exons and 4 introns and encodes 398 amino acids[4].

In 2001, KISS1receptor (GPR54) was paired with the KP by three different groups[2-4]. Tissue distribution of the metastin receptor and its cognate ligand precursor, KISS1, often coincide. Interestingly, transcripts of both are highest in placenta[1,3,4]. Additionally, both KISS1 and the metastin receptors are widespread throughout the central nervous system[4].

High levels of metastin are reported in hypothalamus and pituitary[2], while immunohistochemistry localizes the receptor to neurons in cerebellum, cerebral cortex, thalamus and pons-medulla[4]. KISS1 is also moderately

Abbreviations: KP, kisspeptin protein; Kiss-1, kisspeptin gene; KISS-1, kisspeptin protein; KP-54, a 54 aminoacid peptide of KISS-1 termed as metastin or kisspeptin-54; GPR54, G-protein coupled receptor of kisspeptin-54 or metastin; KISS1 kisspeptin receptor KISS1R, KISS1 receptor gene; MAPK, mitogen –activated protein kinase; ERK, extracellular signal- regulated kinase

expressed in testes, pancreas, liver and small intestine[3,4]. Meanwhile, in addition to placenta, the receptor is also highly expressed in spleen, peripheral blood lymphocytes, testes, lymph nodes, pituitary gland and adipose tissue[3,4].

Downstream signaling of the KISS1 (GPR54) activation

Activation of the KISS1 (GPR54) results in intracellular calcium mobilization that is not affected by pertussis toxin and does not result in changes in cAMP accumulation, suggesting that is a Gq-coupled receptor[2,4]. Numerous studies have sought to further elucidate the downstream signaling pathways activated via stimulation of KISS1 by KP. However, precise mechanisms remain controversial.

At the top of this cascade, KP activation of KISS1 has been shown to simultaneously result in release of arachidonic acid[2] and stimulation of the mitogen-activated protein kinase (MAPKs) extracellular signal–regulated kinase (ERK) 1 and ERK2 kinase[2,6-10]. This has been attributed to increased phosphorylation of MAPK. Additionally, other kinases are reported to be activated by KISS1 including p42/44, PKC, myeloid cell leukemia 1, calcium/ calmodulin-dependent kinases and tyrosine kinases[9,10].

KP and matrix metalloproteinases

Downregulation of one or both of the gelatinase matrix metalloproteinases (MMPs) MMP-2 and MMP-9, by kisspeptins has been shown[6,11-13]. KP have been described as regulators of MMPs at both the transcriptional and protein level. Significantly, active MMPs can cleave the Glycerine-Leucine bond of KP, resulting in the removal of the C terminal three amino acids, leading to inactivation of KP. This may represent a regulatory feedback mechanism between KP and MMPs[14].

Role in cancer metastasis

Because no in vitro assays adequately model tumorigenesis and metastasis, the role of metastin and its receptor in KISS1-mediated metastasis suppression must be examined in vivo.

One strategy for identifying genes involved in metastasis is to inject genes of interest into highly metastatic cells lines and observe changes in their ability to metastasize when injected into athymic nude mice.

The role of KISS1 as a mediator of melanoma metastasis suppression was identified during continued characterization of the metastasis suppression observed following transfer of an intact copy of chromosome 6 into the C8161 human melanoma model. Transfer of normal human chromosome 6 into metastatic malignant melanoma cell lines suppressed metastasis by 95% in this model, without affecting tumorigenicity or local invasiveness[15,16].

The genes, responsible for this phenotype, were identified on chromosome 6 using a modified subtractive hybridization method[16]. A number of candidate genes being identified but only one gene, kiss-1, was expressed in non-metastatic cells and was absent from the metastatic parental line[15]. Kiss-1 maps to chromosome 1, suggesting that the element causing inhibition of metastasis on chromosome 6 may be an important regulator of the KP.

Upstream regulators of KP mediated inhibition of metastasis

Following the identification of a regulatory role for chromosome 6 on kiss-1, research has sought to determine the exact gene responsible. Within region 6q16.3-q23, a number of genes including kinases, vitamin D receptor interacting proteins and arginase-1 were identified[17]. Additionally, direct interaction of Kiss-1 has been identified, which involves regulation of tumorigenesis, metastasis and development[18].

It remains to be determined if KP inhibit cell invasion by altered cell motility, altered adhesiveness, or a combination of both. Recently[10], microarray analysis in the human mammary carcinoma cell line has been used to identify upregulation of a number of genes involved in cell cycle control and apoptosis by KP.

Clinical evidence for a role in cancer

In order to confirm importance of the KP as regulators of metastatic potential in a variety of cancers, changes were detected in KP and KISS1 (GPR54) in native cancer cells from metastatic and non-metastatic tumors. The majority of these reports decreased expression of KP in primary and metastatic tumors, with some reporting a complete absence of KP in metastases[19,20].

While loss of KISS1 expression has been correlated with metastasis, in other studies[3,7] upregulation of the metastin receptor was evident in tumors. Metastin receptor was increased in 10 of 13 papillary carcinomas and not detected in unmatched normal tissues[7].

Correlation of the histopathological stage of tumors with KP expression has shown that peptide levels decrease with progression of the cancer. High expression has been detected in benign and radial growth phase tumors, with lower expression detected in more advanced clinical stages[20-22]. These data supply preliminary evidence supporting a role of these molecules in clinical cancer biology.

Evidence to date has implicated several pathways and suggested endocrine, paracrine and autocrine roles for KISS1 and the metastin receptor and provided a fresh perspective for elucidating KISS1 function. However, the effects of metastin treatment have been observed only in cells expressing the metastin receptor.

Role in placentation

Two-site enzyme immunoassay detected normal plasma KP-54 concentrations of approximately 1 fmol/ml in males and females. Measurement of KP-54 throughout pregnancy revealed 1000-fold increase of KP-54 in the

first trimester, with up to a 10000-fold increase by the third trimester. Levels returned to near baseline by day 5 after partum, suggesting the placenta as the source of KP in pregnancy[23]. Quantitative PCR and microarray analysis detected expression of KP and KISS1 in human trophoblasts[11,24]. Laser capture microdissection specifically detected KP and KISS1 in villous cytotrophoblasts. Transcriptional expression of KP did not change between early and term placentas, but KISS1 expression was higher in early placentas compared to term placentas coinciding with changes from highly invasive cells early in pregnancy to less invasive cells at term[24]. KISS1 expression was localized to villous and extravillous trophoblasts, with KP only expressed in villous trophoblasts, suggesting both autocrine and paracrine actions[11].

Trophoblast migration was inhibited by KP and was also associated with suppression of MMP-2 and MMP-9 activity[11,13]. Immunohistochemical staining of KP-54 in human placenta, detected KP-54-like immunoreactivity in the transport trophoblast subtype, the syncytiotrophoblasts, which are responsible for secretion of peptides into the maternal bloodstream.

The role of KP in placentation and the regulatory association with MMPs led to the hypothesis that they may have an additional role in the pathogenesis of preeclampsia[13].

Role in puberty

Normal puberty requires the integration of central and peripheral signals that converge upon the release of gonadotropin-releasing hormone (GnRH). GnRH is the key hypothalamic hormone regulating reproduction. Pulsatile secretion of GnRH from the hypothalamus triggers episodic gonadotropin secretion from the pituitary, sex steroid secretion from the gonads and gametogenesis. The hypothalamus begins its intermittent secretion of GnRH during fetal development. After the first 6-12 months of human life, the GnRH pulse generator is dampened for several years, a time period known as the childhood quiescence. This interval is followed by a resurgence of the GnRH signal at the expected time of sexual maturation. What signals this resurgence is one of the central questions in reproduction.

Several reproductive disorders, seen commonly in clinical practice, are characterized by abnormalities in the pattern of GnRH secretion. This includes hypothalamic amenorrhea and polycystic ovary syndrome. However, the severest perturbation of GnRH secretion occurs in idiopathic hypogonadotropic hypogonadism (IHH), a disorder characterized by a failure to initiate puberty and permanent sexual infantilism/infertility, if treatment is not initiated. The IHH pathophysiologic model is a unique one to study to mechanisms influencing the developmental fate and function of GnRH neurons.

In 2003, three different groups identified KISS1 as an unexpected molecular switch for puberty[25-27]. Genetic linkage analysis on a consanguineous family, with members who had idiopathic hypogonadotrophic hypogonadism identified a homozygous leucine to serine mutation in the receptor gene[27]. Subjects with these mutations have low gonadotrophin levels and a complete or partial absence of luteinizing hormone (LH) pulsations and do not undergo puberty, although they do respond to treatment with GnRH replacement[27,28].

In parallel to human studies Seminara et al[29] generated KISS1-/- mice. Male mice had greatly reduced testes size, hypoplastic Leydig cells, spermatogenic arrest and lacked development of secondary sex glands. Female mice had small vaginal openings, were sterile and the estrous cycle was absent. Ovary size and uterine horns were greatly reduced and ovaries contained only early follicles, no Graafian follicles or corposa lutea.

A third group[26] studying KISS1 and puberty simultaneously developed knock out mice, which exhibited the same phenotype as those used by Seminara et al. Hormone profiling of these detected striking similarities to the human syndrome, with low gonadotropin levels, but retaining the ability to respond to exogenous GnRH. This shows that whilst the hormone KP is essential for puberty to occur, its absence does not cause any developmental defect in reproductive organs, and that the gonads and pituitary gland are still able to respond to stimulation.

In mice that lack GPR54 the anatomy and localization of GnRH neurons is unremarkable and similarly the amount of GnRH in the brain is not different from that of normal mice. This suggests that there is no defect in the actual synthesis of GnRH, but that somehow, its release from the brain is impaired, and it cannot stimulate the pituitary gland adequately. The KISS1 (GPR54) knockout mice provided an elegant example of a phenocopy syndrome between humans and mice[30]. These results led to the hypothesis that KP has an effect on secretion or processing of GnRH.

KP and GnRH release

Approximately 75% of GnRH neurons co-express KISS1[31], a finding that has been confirmed in sheep, where intracerebral injection of KP resulted in direct release of GnRH into the cerebrospinal fluid[32]. Prominent regions of KP expression in the brain are the arcuate nucleus (Arc), periventricular nucleus (PeN), anteroventral periventricular nucleus (AVPV), with lower levels in the anterodorsal preoptic area, and bed nucleus of the stria termainalis[33-35].

Navarro et al.[33], showed that the total KP and KISS1 mRNA levels in female and male rat hypothalamus are inhibited by oestrogen and testosterone, respectively. The oestrogen receptor-β (ER-β) was not shown to have any role in the KP cascade. In ovary intact, ovariectomized and ovariectomized plus oestrogen-treated female mice, the same pattern as in male mice was observed in the Arc-raised KP in ovariectomized mice that was rescued by oestrogen treatment[34].

Direct evidence for KP as a molecular switch for puberty

Evidence of the activation of the GnRH cascade by the

KP and feedback on the expression of KP in the forebrain by gonadal steroids strongly indicate a role in puberty. To expand on these findings, the role of KP as gatekeepers of puberty has been directly investigated[36].

The pattern of KISS-1 and GPR54 mRNAs has been examined across development in the rat hypothalamus. Upregulation of KP in puberty has been shown in the hypothalamus and AVPV of rats and mice[33,37] and hypothalamus of monkeys[38]. Transcript expression of both the ligand and the receptor shows variation across the estrus cycle with highest levels occurring in diestrus. Central administration of KP to prepubertal female rats advanced vaginal opening, suggesting KP could directly initiate the onset of puberty.

Although the KP pathway is a prerequisite for puberty, it is unlikely to be the sole gatekeeper, requiring interaction with numerous other factors for puberty to commence[36].

KP and estrous cycle

The stimulation of the gonadotrophin axis by the KP suggested possible involvement in the positive feedback loop between oestrogen, GnRH and LH and regulation of the menstrual cycle. Subcutaneous administration of KP-54 induced ovulation in prepubertal female rats, which had been treated with gonadotrophin to induce follicle maturation[39]. Functionally, injection of KP throughout the estrous cycle induced LH secretion and maximal responses were achieved at estrus[40,41].

Increased KP/KISS1 expression is critical for positive feedback in the GnRH cascade and for ovulation[42]. Subsequently, it has been suggested that expression of KP/Kiss1 in the AVPV mediates the process of the GnRH surge at proestrus and ovulation, whereas KISS1 neurons in the Arc are likely to play role in the negative feedback regulation of GnRH/ gonadotrophin secretion[34]. A population of oestrogen-sensitive neurons in the AVPV communicating directly with GnRH neurons was found by Wintermantel et al.[43], a finding which reinforces the above suggestion.

The observation that KISS1 expression is increased in the AVPV at the time of the GnRH/LH surge, coupled with the abolition of the proestrus LH surge by the immunoneutralization of local metastin action in the preoptic area, leads to the conclusion that KISS1 neurons in the AVPV drive the event of ovulation.

KP immunoreactivity was identified in specific ovarian compartments including theca of growing and pre-ovulatory follicles, theca and granulose-lutein cells of corpus luteum and interstitial gland, the ovarian surface epithelium and the oocyte. Interestingly, the staining pattern also changed along the estrous cycle, with the absence of expression in estrous to early proestrous granulosa cells, but detection in late proestrous granulosa cells[40].

The MMPs also have differential expression along the estrous cycle, facilitating follicular breakdown during the periovulatory period. A possible mechanism of the direct action of KP on ovaries is inhibition of MMPs, to prevent unregulated proteolysis of remaining ovarian tissue after follicular breakdown[40].

Direct effects of KP on the testes

In addition to the indirect effects the KP have on the testes via GnRH expression, direct effects on the testes have been shown[23,26,44]. Continuous chronic administration of KP in male rats resulted in decreased testicular weight and degeneration of the seminiferous tubules, leading to the hypothesis that KP may alter testicular blood flow[44].

Role in metabolic syndrome and cardiovascular disease

In women of reproductive age, polycystic ovarian syndrome (PCOS) is a common syndrome, which is associated with infertility, increased LH levels and increased resistance to insulin. Therefore, Panidis et al.[45] decided to investigate potential correlation between PCOS and the KP by comparing KP levels of normal weight women with PCOS, obese women with PCOS and obese controls. In this study, normal weight women with PCOS had significantly higher KP-54 levels and were less insulin resistant than obese women with PCOS. Plasma KP levels were also negatively correlated with body mass index and indices of insulin resistance.

KP and KISS1 (GPR54) have been detected in pancreas[2-4], which is a key regulator of whole-body homeostasis. Both of KP and KISS1 were detected in mouse islets of Langerhans in a- and b-cells, but not in the exocrine pancreatic cells[46].

In functional experiments, exposure of human and mouse islets to KP did not affect basal rate of insulin secretion, but caused a stimulation of glucose-induced insulin secretion.

As hypogonadism is common in uncontrolled diabetes, a further study sought to elucidate the potential role of KP in this phenomenon. In streptozotocin-induced diabetic rats, where the gonadotropic axis is attenuated, KP administration evoked LH and testosterone bursts[47,48]. Kiss1 gene expression was severely decreased in these rats and could be reduced by infusion of leptin, but not insulin.

Leptin is a satiety factor, which is produced by adipocytes and acts on the forebrain, including in the Arc where expression of KISS1 (GPR54) has been described[47,48]. KP reduced the decline in gonadotrophin secretion observed in rats treated with leptin antibodies[47]. Comparison of castrated wild-type mice to leptin-deficient mice identified a significant reduction in KP mRNA levels in the Arc of leptin deficient mice. When treated with leptin KP mRNA levels were increased, although not fully restored[48]. Additionally, 40% of KP mRNA expressing cells in the Arc also expressed the leptin receptor mRNA, suggesting that leptin is a direct regulator of KP neurons.

The endogenous ligand of the growth hormone secretagogue receptor, ghrelin[49], is a regulator of energy homeostasis and reproduction. Ghrelin has been shown to

suppress LH secretion in both rats and monkeys. Therefore, to further elucidate effects of ghrelin on LH secretion, KP and ghrelin were co-administrated. Although ghrelin did not significantly modified the magnitude of the acute stimulatory effects of KP on LH secretion, it did significantly shorten the duration and net magnitude of the response[50].

Diabetes and obesity are associated with cardiovascular disease and, furthermore, tumor metastasis and placentation are processes involving angiogenesis, leading to the hypothesis that the KP may function as novel cardiovascular transmitters. In human, vasculature KISS1 (GPR54) has a restricted localization to smooth muscle of vessels with the same developmental origins, umbilical vein, coronary artery and aorta[51]. Interestingly, KISS1 and KP are also localized to cells within the atherosclerotic plaque of coronary artery and have been shown to act as vasoconstrictors. These suggest that they may act as novel paracrine vascular transmitters at the KISS1 (GPR54) receptor.

Discrete localization of receptor to vessels prone to atherosclerosis also implicates this receptor system in the pathophysiology of cardiovascular disease[51].

Synthetic agonists

It has been revealed that the final five amino acids of KP-10 are essential for agonist activity at KISS1 (GPR54). Synthetic derivatives based on the structure of KP-10, of approximately five amino acids length, showed high affinity and comparable potency to KP-10.

Small molecule compounds with agonist activity at KISS1 (GPR54) were discovered, however they had lower potency and affinity than the native peptide[52,53]. No antagonists at this receptor have currently been described.

Conclusions

The KISS1 receptor (previously designated GPR54) has been paired with biologically active cleavage peptides of the kiss-1 gene product, the kisspeptins (KP). Identification of KISS1 receptor as a molecular switch for puberty subsequently led to the discovery that KP activate the GnRH cascade. Prior to the role of KISS1 in puberty being described, KP had been shown to be inhibitors of tumor metastasis across a range of cancers. PCR detected higher expression of KP and KISS1 receptor in placenta, and changes of KP levels throughout pregnancy and expression in trophoblasts suggests a role in placentation. A role for KP has also been shown in whole body homeostasis.

KP are multifunctional peptides and further investigation is required to fully elucidate the complex pathways regulated by these peptides and how these pathways integrate in the whole body functions.

Synthetic agonists targeting KISS1 receptor may represent novel therapeutic agents for the treatment hypogonadotrophic hypogonadism in some affected individuals.

KP have cast new light on the pathway in the regulation of pubertal timing and reproductive activity.

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